Archives of Gynecology and Obstetrics (2019) 299:211–216
https://doi.org/10.1007/s00404-018-4933-z

GYNECOLOGIC ONCOLOGY

Mesothelin as a target for cervical cancer therapy

Korinna Jöhrens[1] · Lea Lazzerini[2] · Jana Barinoff[2] · Jalid Sehouli[2] · Guenter Cichon[2]

Received: 6 July 2018 / Accepted: 4 October 2018 / Published online: 15 October 2018

Abstract

Purpose The cell surface glycoprotein Mesothelin is overexpressed in several tumor entities and novel immune-based therapies are currently under the early clinical evaluation for the treatment of malignant pleura mesothelioma, ovarian cancer, and pancreatic cancer. Cervical cancer has not been recognized as a suitable target for Mesothelin-directed immune therapies so far.

Methods To exploit a possible role of Mesothelin in cervical cancer treatment, we analysed Mesothelin expression in 79 cervical carcinomas and aligned expressions patterns with tumor growth parameters. A novel anti-Mesothelin drug conjugate (Anetumab Ravtansine) was applied for dose-efficiency studies in a Mesothelin positive tumor model for cervical cancer in Scid mice.

Results In more than three-quarters (77%) of cervical adenocarcinomas, Mesothelin was expressed to high levels. Among squamous cell carcinomas of the cervix uteri expression levels were lower and expression patterns were less intense, but still ranged between 50–60% (57%). A significant correlation between Mesothelin expression levels and tumor grade, metastatic behaviour, and lymph- or hemangiosis was not found. The novel anti-Mesothelin-drug conjugate (Anetumab Ravtansine) showed a substantial dose-dependent therapeutic efficiency in a xenotransplant model for cervical cancer in SCID mice (hela cell tumors). Applying the ADC at a dose of 10 mg/kg twice weekly induced complete tumor regression in 88% of animals within 6 weeks.

Conclusions Mesothelin should be taken into account as a target in cervical cancer therapy and histological determination of Mesothelin expression should be considered in routine diagnostics of cervical carcinomas.

Keywords Mesothelin · Cervical cancer · Cervical adenocarcinomas · Anetumab ravtansine

Introduction

Mesothelin

Mesothelin is a 40 kDa cell surface glycoprotein which is predominantly expressed in mesothelial cells (pleura, pericardium, and peritoneum) [1, 2]. The physiologic function of Mesothelin is not clear. Mesothelin is known to be a binding partner of MUC16 (Ca 125) which suggests a role in cell adhesion; however, it remains unclear whether overexpression of Mesothelin provides a growth advantage to malignant tumors. Mesothelin knock-out mice exhibit a normal phenotype and healthy offspring, which mostly excludes an essential function for this protein [3].

Mesothelin-targeted therapies

The initial Mesothelin-directed clinical studies focused on SS1P and MORAB-009 (Amatuximab). SS1P is a chimeric protein in which the variable region of a Mesothelin-specific antibody was genetically fused to a bacterial toxin (38 kDa portion of *Pseudomonas* exotoxin A). MORAB-009 (Amatuximab) is a chimeric (human–mice) antibody with high affinity to human Mesothelin [4–8]. Binding of MORAB-009 to Mesothelin blocks tumor cell binding to MUC16 inhibits proliferation and elicits cell-mediated cytotoxicity to tumor cells.

✉ Guenter Cichon
guenter.cichon@charite.de

[1] Department of Pathology, Charité-Universitätsmedizin Berlin, Campus Mitte, Charitéplatz 1, 10117 Berlin, Germany

[2] Department of Gynecology, Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany

SS1P and Morab009 were tested as monotherapy and in combination with established chemotherapeutics (pemetrexed/cisplatin) in patients suffering from mesothelioma. The trials were carried out as single arm studies, but, compared to historical data, overall survival in the Amatuximab group was superior by 33% [9]. In 30% of patients treated with SSP1, long-lasting tumor regression was observed [10].

At the time of manuscript preparation, two novel anti-Mesothelin immunoconjugates (Bay94-9343 and BMS-986148) and a therapeutic vaccine (CRS-207) were under early clinical evaluation as the second- and third-line therapies for the treatment of ovarian cancer.

The therapeutic vaccine CRS-207 is a live-attenuated *Listeria monocytogenes* bacteria expressing human Mesothelin vaccine gene. Infused live-attenuated Listeria is taken up by liver phagocytes which then elicit a subsequent inflammatory response and recruitment of natural killer and cytotoxic T-cells. In humans, application of CRS-207 induces a Mesothelin-specific T-cell response in up to 37% of treated patients [11]. In phase I/II studies in patients suffering from Mesothelin overexpressing pancreatic carcinoma, the effect of allogenic granulocyte–macrophage colony-stimulating factor (GM-CSF)-secreting pancreatic tumor cells (GVAXpan) was studied as monotherapy and in combination with CRS-207. Combining Mesothelin vaccine (CRS-207) with the growth factor-secreting tumor cell treatment doubled overall survival rates in comparison to monotherapy of GVAXpan [11].

Anetumab ravtansine on hela cell xenograft tumors

Anetumab ravtansine is a potent antibody-drug conjugate consisting of a human anti-Mesothelin antibody conjugated to the Maytansinoid Tubulin inhibitor DM4 [12]. The antibody binds specifically to human Mesothelin, thereby inducing antigen internalization and disruption of microtubule assembly. This process results in the inhibition of cell growth and cell death of Mesothelin-expressing tumor cells [12]. In this study, we proved the therapeutic efficiency of anetumab ravtansine on hela cell xenotransplant tumors in Scid mice. Hela cells are probably the best known and most widely spread cervical cancer cell line which was established from a 31-year-old cervical cancer patient in 1951 in Baltimore (Maryland, USA). Hela cells express HPV18 oncogenes (HPV E6/7), which are tumorigenic in Scid mice, and xenograft tumors express increased amounts of Mesothelin, which makes them a representative target for Mesothelin-directed therapies.

Over the last 15 years, there has been no progress in cervical cancer therapy and mortality rates in industrial nations remain about 30% (in developing countries about 60–70%). Therefore, searching for novel, safe, and efficient therapeutic strategies is an urgent matter in gynecological oncology.

Materials and methods

IHC staining of tumor tissues for mesothelin expression

The study was approved by the Charité ethics committee (reference number ek.217/20). Paraffin-embedded tissue of 53 squamous cell carcinomas and 26 adenocarcinomas was retrieved from the archive of the department of pathology of the Charité. All cases were primary diagnosed between 2006 and 2015. Pre-treated cases were excluded. The diagnosis was made according to the WHO and re-evaluated before further investigations. Representative formalin-fixed paraffin-embedded tissue of each case encompassing vital tumor areas was stained with haematoxylin and eosin for the histological tumor evaluation.

The expression of Mesothelin was determined on paraffin tissue sections by IHC staining using a Leica Bond™ polymer fully refine detection system. The employed anti-Mesothelin antibody was purchased from Thermo scientific (clone 5B2, 1: 100, Thermo Scientific MS-1320). Mesothelioma tissue was used as a positive control.

All IHC-stained tumor samples were scored by a certified pathologist who was blinded with respect to informations about the tumor state and outcome. Scoring was performed according to a procedure described by Hirsch et al. [13] as follows:

Three representative high-power fields from each tumor sample were selected and the mean h-score was calculated as the product of relative proportion of Mesothelin positive tumor cells (percent) and IHC staining intensity (1+, 2+, 3+) as follows: $1\times$(% cells 1+) $+2\times$(% cells 2+)$+3\times$(% cells 3+). The highest possible score is 300. According to the score Mesothelin expression was rated to be absent (score 0), low (score 1–9), moderate (score 10–49), and high (score 50–300).

Animal experiments

Experiments were approved by the state office for health and social affairs Berlin (Landesamt für Gesundheit und Soziales (Berlin); reference number G 0262/10. SCID-beige mice were purchased from Charles River Laboratories (Sulzfeld, Germany). Hela cells were derived from the DSMZ (Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany). Anti-Mesothelin Maytansinoid conjugate (Ravtansine Anetumab) was provided from Bayer (Berlin, Germany). Four groups of mice, each consisting of 5–8 animals was set up and vital Hela cells were inoculated in a dose of $1\times10e5$ cells in 100 µl by subcutaneous injection (27 gauge needles) in

the left inner flank on day 0. Treatment was started on day 30 when tumors have reached a size of 5–10 mm. When tumors were grown to a size of about 5 mm treatment was started. Animals in the treatment groups received 2 mg/kg, 5 mg/kg, and 10 mg/kg anetumab ravtansine in 200 µl injection buffer twice weekly by i.p. injection. Animals in the control group received injection buffer only. Tumor size was monitored by caliper twice weekly.

Results

About 63% (50/79) of all cervical carcinomas show a positive Mesothelin expression. Considering cervical adenocarcinomas and squamous cell carcinomas separately, it is striking that, in adenocarcinomas, not only the frequency but also the intensity of the Mesothelin expression is markedly increased. 73% (19 out of 26) adenocarcinomas show high levels of Mesothelin (h-score 50–300) and only 23% (6/26) do not show any Mesothelin expression. In cervical squamous cell cancer, about 57% (30/53) are stained positive for Mesothelin and expression patterns are less pronounced compared to adenocarcinomas (Table 1).

Correlating the Mesothelin expression with the nodal status, tumor grade and the frequency of blood and lymph vessels infiltration (L/V) did not reveal a significant correlation. Distribution of Mesothelin expression patterns appears completely random (Table 2).

Already in preinvasive lesiones of the cervix (AIS) an increased epithelial Mesothelin expression is often detectable (Fig. 1a). The majority of cervical adeno- and squamous cell carcinomas shows overexpression of the membrane-bound glycoprotein (Table 1; Fig. 1b, c) as well as hela xenograft tumors (Fig. 1d), which makes the latter an experimental target for Mesothelin directed immunotherapies.

Effect of anetumab ravtansine on hela cell tumors in SCID mice

Tumor-bearing SCID mice received the anti-Mesothelin toxin conjugate (Anetumab Ravtansine) in a dose of 2 mg/kg, 5 mg/kg, and 10 mg/kg twice weekly or PBS only. The application of 2 mg/kg ADC twice weekly slowed tumor growth and prolonged survival time, but did not induce growth arrest or reduction of tumor volume. Applying 5 mg/kg twice weekly healed two of eight animals (25%) and induced complete growth arrest in six animals within 6 weeks. A further dose increase to 10 mg/kg raised the cure rate to 88% (7 of 8). The treatment was tolerated well and animals did not show weight loss or suspect behavioural signs, but some animals developed ulcerations of the tumor covering skin during the treatment (Fig. 2).

Table 1 Frequency and density of Mesothelin expression in cervical adenocarcinomas and squamous cell cancer

	Mesothelin expression absent (*h*-score 0)	Mesothelin expression low (*h*-score 1–9)	Mesothelin expression moderate (*h*-score 10–49)	Mesothelin expression high (*h*-score 50–300)
Cervical adenocarcinomas ($n = 26$)	6/26 (23%)	0%	1/26 (3.9%)	19/26 (73.1)%
Squamous cell cervical cancer ($n = 53$)	23/53 (43.4%)	2/53 (3.8%)	14/53(26.4%)	14/53 (26.4%)

Table 2 Assignment of mesothelin expression and nodal status, differentiation, and lymph- and hemangiosis patterns in cervical squamous cell cancer

Squamous cell cervical cancer ($n = 53$)	Mesothelin expression absent (*h*-score 0)	Mesothelin expression low (*h*-score 1–9)	Mesothelin expression moderate (*h*-score 10–49)	Mesothelin expression high (*h*-score 50–300)
N0	15/53 (28.3%)	0/53 (0%)	9/53 (16.9%)	8/53 (15.1%)
N1	7/53 (13.3%)	3/53 (5.6%)	4/53 (7.5%)	7/53 (13.3%)
G1	1/53 (1.9%)	0/53 (0%)	1/53 (1.9%)	1/53 (1.9%)
G2	14/53 (26.4%)	2/53 (3.8%)	6/53 (11.3%)	9/53 (17%)
G3	9/53 (17%)	0/53 (0%)	5/53 (9.4%)	5/53 (9.4%)
L0	11/53 (20.7%)	1/53 (1.9%)	4/53 (7.5%)	9/53 (17%)
L1	11/53 (20,7%)	1/53 (1.9%)	10/53 (18.7%)	5/53 (9.4%)
V0	18/53 (34%)	1/53 (1.9%)	10/53 (18.9%)	14/53 (26.4%)
V1	4/53 (7.5%)	1/53 (1.9%)	3/53 (5.6%)	2/53 (3.8%)

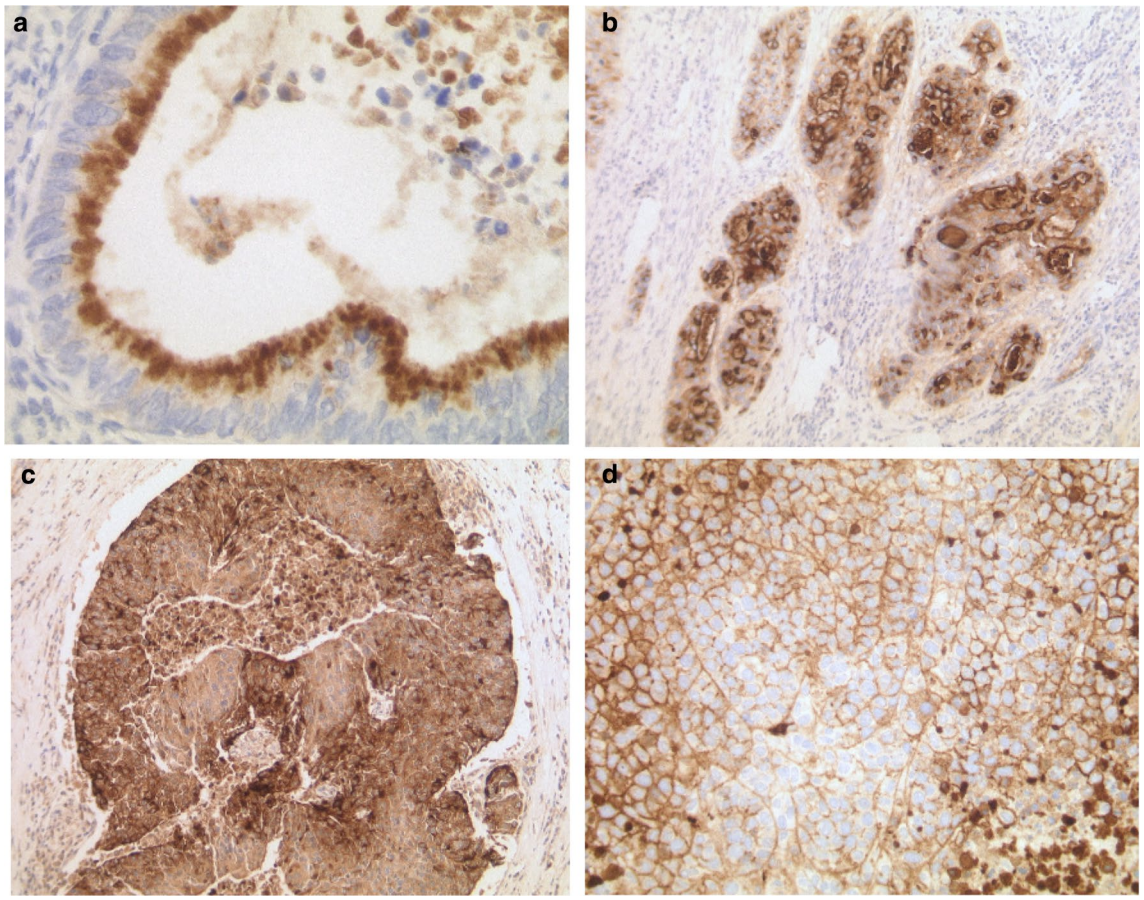

Fig. 1 Mesothelin expression in an adenocarcinoma in situ (**a**), in a cervical adenocarcinoma (**b**), in cervical squamous cell cancer (**c**), and in hela cell xenograft tumors. Mesothelin occurs as a membrane bound protein

Discussion

So far, the early clinical testing of novel Mesothelin-directed immune therapies focus on the treatment of pleuramesothelioma, ovarian cancer, and pancreatic cancer [14]. Cervical cancer has not been recognized as a suitable target disease and only limited information was available about Mesothelin expression in this tumor entity. We here demonstrate that, on average, 60–70% of cervical carcinomas express Mesothelin. Looking at the Mesothelin expressions patterns in squamous cell and adenocarcinomas of the cervix separately reveals significant differences between both subgroups. Cervical adenocarcinomas express Mesothelin not only more frequently, but also the level of expression is markedly higher. This finding is especially interesting in view of the worse prognosis of cervical adenocarcinomas as it may open up new treatment options for this tumor entity.

The role of Mesothelin as a prognostic marker in malignant tumors is discussed controversially. The majority of studies describe increased Mesothelin expression to be a negative prognostic factor in pleuramesothelioma [15], breast cancer [16, 17], lung cancer [18, 19], and gastric cancer [20]. However, the other studies on breast cancer could not find any correlation [21] or even demonstrate an improved prognosis of high Mesothelin expression like in gastric cancer [22].

In this study, no significant correlation was found between Mesothelin expression and the metastatic state (N), tumor grade (G), and blood and lymph vessel infiltration (L/V). Mesothelin expression in squamous cell tumors and adenocarcinomas of the cervix occurred completely random and did not seem to be associated with tumor growth patterns and metastatic behaviour (Tables 2, 3).

The anti-Mesothelin–Maytansinoid conjugate (anetumab ravtansine) is currently applied in the early clinical trials for the treatment of malignant mesothelioma, ovarian cancer, and triple negative breast cancer. In this study, we explored the therapeutic potential of anetumab ravtansine in a murine animal model for human cervical cancer (hela cell tumors in SCID mice). Anetumab ravtansine shows a dose-dependent therapeutic efficacy on hela xenograft tumors. In the highest dose group (10 mg/kg twice weekly) in 7 of 8

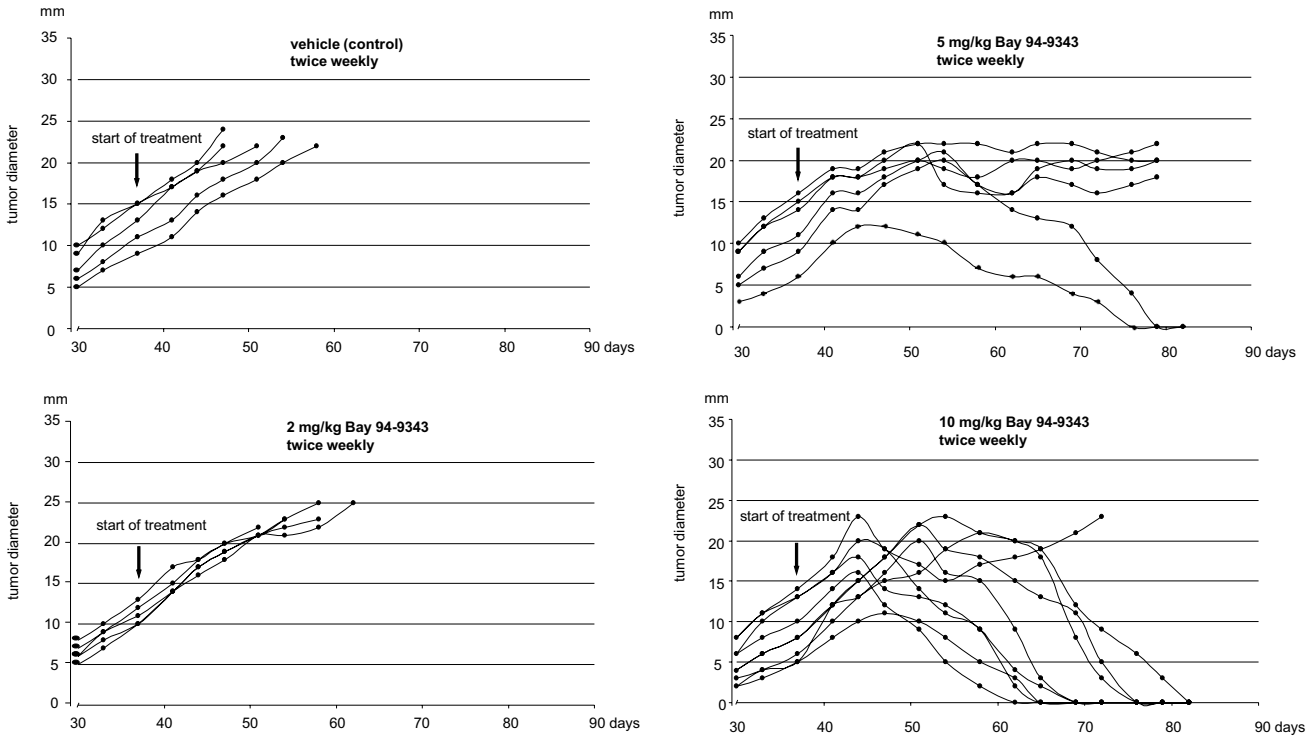

Fig. 2 Effect of anti-Mesothelin–Maytansinoid conjugate (anetumab ravtansine) on hela xenograft tumors in SCID mice. Animals received the ADC up to 6 weeks twice weekly

Table 3 Assignment of Mesothelin expression and nodal status, differentiation, and lymph- and hemangiosis patterns in cervical adenocarcinomas

Cervical adenocarcinomas ($n = 26$)	Mesothelin expression absent (*h*-score 0)	Mesothelin expression low (*h*-score 1–9)	Mesothelin expression moderate (*h*-score 10–49)	Mesothelin expression high (*h*-score 50–300)
N0	5/26 (19.2%)	0/26 (0%)	2/26 (7.7%)	15/26 (57.8%)
N1	3/26 (11.5%)	0/26 (0%)	0/26 (0%)	1/26 (3.8%)
G1	0/26 (0%)	0/26 (0%)	0/26 (0%)	3/26 (11.5%)
G2	3/26 (11.5%)	0/26 (0%)	0/26 (0%)	15/26 (57.8%)
G3	3/26 (11.5%)	0/26 (0%)	0/26 (0%)	2/26 (7.7%)
L0	1/26 (3.8%)	0/26 (0%)	0/26 (0%)	13/26 (50%)
L1	6/26 (23.1%)	0/26 (0%)	0/26 (0%)	6/26 (23.1%)
V0	4/26 (15.4%)	0/26 (0%)	0/26 (0%)	20/26 (76.9%)
V1	2/26 (7.7%)	0/26 (0%)	0/26 (0%)	0/26 (0%)

mice (88%), complete regression of large tumors occurred within 6 weeks. Mice tolerated the treatment very well, but, since anetumab ravtansine does binds selectively to human Mesothelin (in hela cell tumors) and not to the murine counterpart, side effects are limited. However, this model demonstrates the therapeutic potential of this antibody-drug conjugate.

In the early clinical escalation trials, administration of 6.5 mg/kg every 3 weeks (q3w) has been shown to provide an acceptable benefit–risk ratio. The most common side effects were keratitis and peripheral neuropathy which all were reversible [23, 24]. At a maximum tolerated dose (MTD) of 6.5 mg/kg, partial response (PR) was seen in 19% and stable disease in 47% of patients.

In summary, the high expression level of Mesothelin in cervical adenocarcinomas favours this tumor entity as a target for novel Mesothelin-directed immune therapies. Affected women should be included into the early clinical studies and Mesothelin staining should be considered as a standard procedure in clinical routine.

Acknowledgements This study was funded by the Bundesministerium für Bildung und Forschung (BMBF) 03V0347 and the Bayer AG. The authors would like to thank Natalie Kuzaj and Andrea Wilke for the excellent technical support.

Author contribution KJ investigation and methodology; LL methodology; JB writing, review, and editing; JS supervision; GC conceptualization, writing, review, and editing.

Compliance with ethical standards

Conflict of interest Dr. Cichon received grant monies from Bayer AG. Prof. Sehouli receives funding for studies in ovarian cancer and serves on the Advisory Board as a consultant for Bayer. The other authors have no conflict of interest to declare.

References

1. Chang K, Pastan I, Willingham MC (1992) Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. Int J Cancer 50:373–381
2. Einama T, Kawamata F, Kamachi H, Nishihara H, Homma S, Matsuzawa F et al (2016) Clinical impacts of mesothelin expression in gastrointestinal carcinomas. World J Gastrointest Pathophysiol 7:218–222
3. Bera TK, Pastan I (2000) Mesothelin is not required for normal mouse development or reproduction. Mol Cell Biol 20:2902–2906
4. Reiter Y, Pastan I (1996) Antibody engineering of recombinant Fv immunotoxins for improved targeting of cancer: disulfide-stabilized Fv immunotoxins. Clin Cancer Res 2:245–252
5. Hassan R, Bullock S, Premkumar A, Kreitman RJ, Kindler H, Willingham MC et al (2007) Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus IV infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. Clin Cancer Res 13:5144–5149
6. Hassan R, Sharon E, Thomas A, Zhang J, Ling A, Miettinen M et al (2014) Phase 1 study of the antimesothelin immunotoxin SS1P in combination with pemetrexed and cisplatin for front-line therapy of pleural mesothelioma and correlation of tumor response with serum mesothelin, megakaryocyte potentiating factor, and cancer antigen 125. Cancer 120:3311–3319
7. Hassan R, Ebel W, Routhier EL, Patel R, Kline JB, Zhang J et al (2007) Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. Cancer Immun 7:20
8. Hassan R, Cohen SJ, Phillips M, Pastan I, Sharon E, Kelly RJ et al (2010) Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. Clin Cancer Res 16:6132–6138
9. Hassan R, Kindler HL, Jahan T, Bazhenova L, Reck M, Thomas A et al (2014) Phase II clinical trial of amatuximab, a chimeric antimesothelin antibody with pemetrexed and cisplatin in advanced unresectable pleural mesothelioma. Clin Cancer Res 20:5927–5936
10. Hassan R, Miller AC, Sharon E, Thomas A, Reynolds JC, Ling A et al (2013) Major cancer regressions in mesothelioma after treatment with an anti-mesothelin immunotoxin and immune suppression. Sci Transl Med 5:208ra147
11. Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G et al (2015) Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol 33:1325–1333
12. Golfier S, Kopitz C, Kahnert A, Heisler I, Schatz CA et al (2014) Anetumab ravtansine: a novel mesothelin-targeting antibody-drug conjugate cures tumors with heterogeneous target expression favored by bystander effect. Mol Cancer Ther 13(6):1537–1548
13. Hirsch FR, Varella-Garcia M, Bunn PA Jr, Di Maria MV, Veve R, Bremmes RM et al (2003) Epidermal growth factor receptor in non-small-cell lung carcinaoms: correlation between gene copy number and protein expression and impact on prognosis. J Clin Oncol 21(20):3798–807
14. Zhao XY, Subramanyam B, Sarapa N, Golfier S, Dinter H (2016) Novel antibody therapeutics targeting mesothelin in solid tumors. Clin Cancer Drugs 3:76–86
15. Tian L, Zeng R, Wang X, Shen C, Lai Y, Wang M et al (2017) Prognostic significance of soluble mesothelin in malignant pleural mesothelioma: a meta-analysis. Oncotarget 8(28):46425–46435
16. Wang Y, Wang L, Li D, Wang HB, Chen QF (2012) Mesothelin promotes invasion and metastasis in breast cancer cells. J Int Med Res 40:2109–2116
17. Tozbikian G, Brogi E, Kadota K, Catalano J, Akram M, Patil S et al (2014) Mesothelin expression in triple negative breast carcinomas correlates significantly with basal-like phenotype, distant metastases and decreased survival. PLoS One 9:e114900
18. Kachala SS, Bograd AJ, Villena-Vargas J, Suzuki K, Servais EL, Kadota K et al (2014) Mesothelin overexpression is a marker of tumor aggressiveness and is associated with reduced recurrence-free and overall survival in early-stage lung adenocarcinoma. Clin Cancer Res 20(4):1020–1028
19. He X, Wang L, Riedel H, Wang K, Yang Y, Dinu CZ et al (2017) Mesothelin promotes epithelial-to-mesenchymal transition and tumorigenicity of human lung cancer and mesothelioma cells. Mol Cancer 16:63
20. Han SH, Joo M, Kim H, Chang S (2017) Mesothelin expression in gastric adenocarcinoma and its relation to clinical outcomes. J Pathol Transl Med 51:122–128
21. Parinyanitikul N, Blumenschein GR, Wu Y, Lei X, Chavez-Macgregor M, Smart M et al (2013) Mesothelin expression and survival outcomes in triple receptor negative breast cancer. Clin Breast Cancer 13:378–384
22. Baba K, Ishigami S, Arigami T, Uenosono Y, Okumura H, Matsumoto M et al (2012) Mesothelin expression correlates with prolonged patient survival in gastric cancer. J Surg Oncol 105:195–199
23. Bendell J, Blumenschein G, Zinner R, et al. (2016) First-in-human phase I dose escalation study of a novel anti-mesothelin antibody drug conjugate (ADC), BAY 94-9343, in patients with advanced solid tumors. In: Proceedings of the 104th annual meeting of the American 86 clinical cancer drugs 3; no. 2
24. Hassan et al. (2015) Phase I study of anti-mesothelin antibody drug conjugate anetumab ravtansine (ID 1574; oral presentation). In: 16th World Conference on Lung Cancer WCLC 2015 in Denver, Colorado, September 6–9, 20